LIST OF PUBLICATIONS


Comparison of plasma prolactin and CEA in monitoring patients with adenocarcinoma of colon and rectum


Division of Research, Gujarat Cancer Society, Asarwa, Ahmedabad 380 016, India.

Summary Plasma prolactin (PRL) and carcinoembryonic antigen (CEA) were measured by radioimmunoassay in 74 patients with adenocarcinoma of colon and rectum. The markers were correlated with disease stage, histological grade and progression/remission of disease. The circulating preoperative median PRL and CEA levels were significantly higher in colorectal cancer patients than in their respective controls. PRL was elevated in all Dukes stages and in all histological grades of the tumour whereas the rise in CEA was more pronounced in Dukes D. Out of 74 patients, 29% (21/74) developed recurrent disease and 31% (23/74) responded to the treatment. With regard to monitoring recurrence(s), the predictive value of PRL was 94% which was significantly greater than that of CEA which was only 62%. In patients who developed liver metastases PRL remained elevated whereas CEA showed more than 100-fold increase. Therefore, we feel that CEA is a better marker for monitoring patients who developed liver metastases. From our results, we suggest that PRL can be used as a better overall marker for detecting recurrence(s) in patients with colorectal adenocarcinoma.

Materials and methods

Patients

Seventy-four colorectal cancer patients treated at The Gujarat Cancer & Research Institute, Ahmedabad, India were included in the study between January 1987 to July 1991. There were 46 males, ten premenopausal and 18 postmenopausal females. Age matched healthy controls of either sex (n = 50) were also examined. Only those females who had ceased to menstruate for 5 years were regarded as postmenopausal.

Blood collection

Blood samples were collected in EDTA, disodium salt coated tubes (1–2 mg ml⁻¹) for prolactin (PRL) and CEA estimations strictly between 9.0 and 11.0 a.m. preoperatively and at monthly intervals thereafter. The plasma was separated within 1–2 h of collection, aliquoted and stored at −70°C. Assays were carried out within 1 month of collection.

Pathological examination

Disease was staged using Dukes system (Dukes & Bussey, 1958). The histologic grades were assessed independently by two histopathologists who were unaware of other parameters.

Therapy

The primary treatment offered to the patients was surgery (curative resection-Dukes A to C). Operative findings were noted of all the patients. Postoperative radiotherapy and/or chemotherapy was instituted. Patients with Dukes C and D received chemotherapy (5 FU, n = 47). The treatment was planned by clinical oncologists of our institute.

Assessment of disease activity

The preoperative assessment was done using standard methods viz. sigmoidoscopy, barium enema, chest X-ray, abdomino-pelvic ultrasonography and biochemical tests for liver and renal functions. During follow-up the patients underwent clinical and biochemical examinations complemented, if necessary, with radiologic, ultrasonographical and fine needle aspiration cytology.

Seventy-four patients were initially included in the study, however, at the end of 2 years, 23 patients responded to the treatment, 21 developed recurrence and rest were lost to follow-up. In patients who developed recurrence, 1/21 (5%) each had Dukes B & D whereas 19/21 (90%) had Dukes C disease. 15/21 (71%) developed local recurrence, 3/21 (14%) developed liver metastases, 2/21 (10%) developed bone and 1/21 (5%) developed lung metastases.

Plasma PRL and CEA were assayed using double antibody RIA kits (Diagnostic Products Co., USA). The assays were performed in duplicate with an intra- and inter-assay coefficient of variation (CV) of 3–5% and 5–8% respectively. PRL values >15.0 ng ml⁻¹ for males, >20.0 ng ml⁻¹ for premenopausal and >10.0 ng ml⁻¹ for postmenopausal females were considered for % elevation. CEA levels above 5.0 ng ml⁻¹ was regarded as % elevated.

Criteria for positive tests were: continual rise in the marker level after an initial fall or persistent high level of the marker as an indicator of relapse and/or no response to treatment.

Statistical analysis

The statistical significance of differences between various groups was calculated by Mann-Whitney U-test. α value
<0.05 (two tailed test) were considered statistically significant. Karl-Pearson correlation coefficient (r) was used to calculate correlation between two parameters. Sensitivity, specificity and predictive values were calculated as described by Tondini et al. (1988).

Results

Preoperative plasma PRL and CEA levels for controls and colorectal cancer patients are shown in Table I. No correlation was observed between two markers (r = + 0.037). Median marker levels were significantly elevated in colorectal carcinoma patients. Table II shows the distribution of patients according to Dukes stages. Sixty-three percent of our patients had advanced disease (C and D). Median PRL level in male patients was higher in Dukes B and C than in D (Figure 1). Dukes D patients showed higher CEA levels than A, B and C (Figure 2).

The median levels of PRL and CEA were more or less similar in all the three grades of the tumour. This may be due to the fact that 91% patients had histologic grade II and III tumour.

Markers in responders

All patients who responded to various therapeutic modalities at the end of 2 years showed decreased PRL and CEA levels. The difference was statistically significant only for PRL (Table III). Non-progressive elevation of CEA was seen in 7/23 (30%).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Prolactin and CEA in colorectal carcinoma patients at diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Prolactin</strong> (median, ng ml⁻¹)</td>
</tr>
<tr>
<td>Controls</td>
<td>Males</td>
</tr>
<tr>
<td>Range</td>
<td>2.3-0.098.82</td>
</tr>
<tr>
<td>Colorectal cancer patients</td>
<td>46</td>
</tr>
<tr>
<td>Range</td>
<td>1.2-195.0</td>
</tr>
<tr>
<td>% elevation (above upper normal limit)</td>
<td>97</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mann-Whitney U-test <sup>a</sup><sub><sup>a</sup></sub>\(<0.01</sub>

Markers in patients who developed recurrence

On sequential follow-up, the PRL levels reduced at response whereas with the appearance of local/distant metastases, the PRL levels increased significantly (Table III). It was observed that the rise in PRL preceded disease progression by approximately 2–3 months. Moreover, PRL levels also remained elevated throughout the course of disease in patients who did not respond to adjuvant therapy (Figure 3). On sequential follow-up, CEA levels reduced with remission whereas with appearance of recurrence, the CEA levels increased only in 17/21 (81%) patients (Table III). In patients with Dukes D, as the disease progressed PRL remained elevated but CEA showed remarkable increase (Figure 4).

![Figure 3 Patient had Dukes C grade II tumour and 14/16 metastatic pararectal lymph nodes. Post-operative chemotherapy was given. He responded to it. He was without any complaints for nearly 7 months. At the end of 1st year, he developed lung metastasis. Second line chemotherapy was instituted but he did not respond to it and finally died. PRL showed lead time and correlated with the lowering of PRL levels.](image)

![Figure 4 Dukes D patient with metastasis in the liver. Post-operative CEA decreased while PRL was elevated. She was given palliative CT to which she did not respond. Both the markers correlated well with the disease status. PRL remained elevated whereas 100-fold increase was observed for CEA.](image)

Table IV Sensitivity, specificity and predictive value of PRL and CEA

<table>
<thead>
<tr>
<th></th>
<th>PRL</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94%</td>
<td>76%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96%</td>
<td>65%</td>
</tr>
<tr>
<td>Predictive value</td>
<td>94%</td>
<td>62%</td>
</tr>
</tbody>
</table>

Sensitivity, specificity and predictive values of the markers

Sensitivity, specificity and predictive values of PRL and CEA in monitoring disease course are shown in Table IV. The values were significantly higher for PRL than for CEA.

Discussion

The present study investigated comparison between PRL and CEA levels with disease stage, histologic grade and disease course in patients with colorectal adenocarcinoma. Elevated PRL was found more often in patients with Dukes A to C than D. Six out of ten (60%) premenopausal patients had hyperprolactinaemia which is more frequent in our patients with colorectal cancer. From our results we think that the hormonal abnormalities might be responsible for the development and progression of the disease (Bhatavdekar et al., unpublished data). Dukes D patients had low level of prolactin so, we have estimated PRL in the plasma and ascitic fluid collected simultaneously in a few Dukes D patients. We found significantly higher PRL concentrations in the ascitic fluid compared to the circulating levels. On this basis we presume that PRL, which is a low molecular weight polypeptide (approx. 23,000 dalton) easily escapes into the ascites from the circulation or the lymphatics (Bhatavdekar et al., unpublished data). However, a larger patients series is essential to confirm these preliminary results.

Plasma PRL levels correlated very well with the disease progression. Most of these patients responded to treatment and this was correlated with the lowering of PRL levels. However, PRL levels increased with local/distant metastases. An early rise in PRL in colorectal cancer patients is an important finding and may offer a sensitive means to predict the presence of recurrent disease which is often difficult to evaluate by other means. The rising PRL level is useful in early diagnosis of progressive disease. PRL even showed a lead time of 2–3 months. Thus, serial estimations of rising PRL levels are useful in the early diagnosis of progressive disease.

It was observed that though CEA levels were high in all the Dukes stages and grades of the tumour, no intergroup variation was observed except in Dukes D patients. Regarding sequential estimations of CEA, there is some controversy about the adequacy of CEA as a monitor of disease activity in colorectal cancer. Some studies (Moertel et al., 1978; Ovaska et al., 1990) have found it less sensitive and therefore unsatisfactory whereas Staab et al. (1985) found it quite reliable. The present study, however, suggests that CEA may be of little practical value in local/distant metastases. Even in patients who developed recurrence(s), CEA remained <5.0 ng ml⁻¹ plasma in 24% of patients throughout the course of the disease. In such patients, PRL accurately predicted disease progression (Figure 3). Moreover, temporary, non-progressive elevations of CEA were seen in 30% of patients, which is an extreme example of this phenomenon (Rittgers et al., 1978). Despite the lack of specificity for colon cancer, CEA demonstrated an excellent correlation with patients with colorectal liver metastases (Chu et al., 1982; DeBrauw et al., 1987; Lorenz et al., 1989; Chang et al., 1989). Our study confirms these findings with 100% score. In these patients PRL remained high.

On the basis of the present encouraging results, we support that CEA lacks sufficient sensitivity and specificity to detect occult recurrence(s). CEA is most useful in monitoring...
patients who developed liver metastases. On the contrary, plasma PRL is a very important independent predictor of recurrent disease which may be due to higher sensitivity, specificity and significantly higher predictive values.

The research work was supported by the Indian Council of Medical Research (#8704250) New Delhi, India. The authors are thankful to Dr N L Patel, Director, The Gujarat Cancer & Research Institute for providing necessary facilities.

References


Plasma Prolactin in Patients with Colorectal Cancer
Value in Follow-up and as a Prognosticator


Background. Preoperative plasma prolactin and carcinoembryonic antigen (CEA) levels were assessed to monitor disease recurrence and to identify low-risk and high-risk patients with Dukes B or C colorectal cancer.

Methods. Prolactin and CEA were estimated by radio-immunoassay method. Blood samples were collected preoperatively and sequentially thereafter from patients with colorectal cancer (N = 114); the samples were compared with samples from age-matched healthy control subjects (smokers and nonsmokers, N = 45). For rest of the analysis, patients with Dukes A disease (N = 7) were not included because of the small number. In monitoring recurrences, the criteria for positive test for the two markers was a continual increase in the marker level after an initial decrease or persistent high level of the marker. These were the indicators of relapse or no response to treatment. To determine the efficacy of the preoperative markers, the patients were grouped according to disease status at the end of 3 years, i.e., patients who had response to the treatment modalities (N = 52) and patients who later had progressive disease (N = 55). To determine the prognostic significance of preoperative marker levels, the patients were divided according to the cutoff levels (upper normal limits); for prolactin the cutoff level was 20.0 ng/ml plasma, and for CEA it was 5.0 ng/ml plasma.

Results. Both of the markers were significantly high in patients with colorectal cancer compared with the markers of their respective control subjects (P < 0.0001). In monitoring disease course, the predictive power of prolactin was 100%, whereas that of CEA was 66%. Prolactin showed a lead time of 2–3 months. Preoperative prolactin levels were significantly higher in patients who later had progressive disease (P < 0.001) than in patients who had response to the treatments. However, such an intergroup variation was not observed for CEA. Patients with preoperative levels of prolactin greater than 20.0 ng/ml had shorter overall survival times than did those with prolactin levels less than 20.0 ng/ml plasma; such a trend was not observed for patients with CEA levels less than 5.0 ng/ml and those with CEA levels greater than 5.0 ng/ml plasma.

Conclusion. Prolactin is a better overall marker than is CEA in patients with Dukes B or C colorectal cancer. The authors recommend the use of plasma prolactin levels to help identify low-risk and high-risk patient subgroups so that high-risk patients may be followed up more intensely and treated accordingly. Hyperprolactinemic patients with Dukes B or C disease have shortened survival time. Cancer 1994; 73:570-4.

Key words: prolactin, carcinoembryonic antigen, Dukes B or C, disease monitor, prognosticator.

In colorectal carcinoma, locoregional control remains a major problem among patients with Dukes B or C disease. These patients constitute a high-risk subset and obviously would be candidates for a more effective treatment. Many tests are available that can be used to search for recurrent cancers. However, none of the techniques are of clinical value in early detection of recurrence.

In a preliminary study, we have compared plasma prolactin and CEA levels in 74 patients with Dukes A to D colorectal cancer. The data mainly concern the relationship of these markers with the stage of the disease, histologic grade of the tumor, and their use in monitor-
Hyperprolactinemia and Unfavorable Prognosis/Pafe/ et al. 571

Table 1. Patient Characteristics and Tumor Stage and Location

<table>
<thead>
<tr>
<th>Dukes stage</th>
<th>Males (N)</th>
<th>Females (N)</th>
<th>Age (yr)</th>
<th>Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>0</td>
<td>38-75</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>36</td>
<td>17</td>
<td>25-84</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>31</td>
<td>23</td>
<td>17-77</td>
<td>13</td>
<td>41</td>
</tr>
</tbody>
</table>

ing recurrences. Our result suggests that prolactin levels were high in all Dukes stages and grades of the tumor and can be used as a better overall marker for monitoring recurrences. CEA determination seems to be of little value in colorectal carcinoma but is useful in monitoring colorectal liver metastases. Our data point toward a significant role of prolactin in the pathogenesis of colorectal cancer. The aims of this study were to investigate the usefulness of serial estimation of plasma prolactin and CEA for detection of recurrence or distant metastases and to find a better marker to differentiate low-risk and high-risk subgroups in patients with colon and rectal cancer.

Patients and Methods

A total of 114 patients, registered at The Gujarat Cancer and Research Institute between 1985 and 1989, were included in this retrospective study; of the 114, 74 were male and 40 were female patients. Clinical, pathologic, and biochemical data were evaluated and recorded in 7 patients with Dukes A, 53 patients with Dukes B, and 54 patients with Dukes C carcinoma of the colon and rectum. Ninety-four percent of patients had Grade II/III tumors. All patients underwent surgery, staging was confirmed by pathologic examination of the specimen, and modified Dukes classification was used. Blood samples of the patients were obtained preoperatively and monthly/bimonthly for the first 2 years and every 3 months thereafter. The collection was done between 9:00 and 11:00 a.m. by peripheral venipuncture. Blood was collected in ethylenediamine tetraacetic acid-coated tubes (1-2 mg/ml), plasma was separated within 2 hours and preserved at -70°C until assayed. Prolactin and CEA concentrations were determined by radioimmunoassay using radioimmunoassay kits. The assays were performed in duplicate with an intra- and inter-assay coefficient of variation of 3-8%

The number of patients with Dukes A was only 7, so additional study data include only patients with Dukes B or C cancer (N = 107). All 107 patients were followed up for a minimum of 3 years. Data regarding patient sex and age, Dukes stage, and tumor distribution are presented in Table 1. The criteria for positive tests were a continual increase in the marker level after an initial decrease or persistent high level of the marker. These were the indicators of relapse or no response to treatment.

The treatments were decided by the clinicians of our institute. All of the patients were treated with curative resection. Patients with Dukes B or C cancer were treated with postoperative radiation therapy or chemotherapy. On the basis of disease status the patients were divided into two subgroups. Group 1 was made up of patients who had a response to treatment (inactive disease; N = 52) and patients who were free of disease at the end of 3 years. Group 2 patients later had progressive disease (N = 55) or recurrent disease within 3 years. Of 107 patients, 51% later had progressive disease and 49% had response to the treatment. Of the patients who later had progressive disease, 48 of 55 (87%) died within 3 years.

To determine the prognostic value of the markers, the patients were divided into two subgroups for each marker, namely prolactin levels less than 20 ng/ml and prolactin levels greater than 20.0 ng/ml (hyperprolactinemia); and CEA levels less than 5.0 ng/ml and CEA levels greater than 5.0 ng/ml plasma. These cutoff levels were the upper normal limit of the markers. Significance was calculated, and P values less than 0.05 were considered significant. Spearman's correlation coefficient (r) was used to determine the correlation between two parameters. Survival was calculated according to life-table analysis.

Results

Preoperative plasma prolactin (mean ± standard error, 34.85 ± 5.42 ng/ml) and CEA (mean ± SE, 12.16 ± 1.96 ng/ml) levels of patients with colorectal cancer were significantly higher than those of their respective control subjects (prolactin, 8.89 ± 0.69 ng/ml; CEA, 1.44 ± 0.25 ng/ml; P < 0.0001). No correlation was observed between the two markers (r = 0.08). In patients with Dukes B or C colorectal cancer, 45% had hyperprolactinemia, and CEA was elevated in 65% of patients (Fig 1).

Monitoring Disease Course With Markers

On sequential follow-up, the prolactin levels were significantly reduced in patients with response, whereas patients who later had progressive disease had increased prolactin levels. The increase in prolactin level preceded clinical disease recurrence by 2-3 months. Similar results were not observed for CEA (results not shown). The predictive power of prolactin was 100% and that of CEA was 66% (Fig 2).
Efficacy of Preoperative Markers as Predictors of Response

The patients were divided into two subgroups: those who had response to treatment (N = 52) and those who later had progressive disease (N = 55). Patients who later had progressive disease had significantly higher preoperative prolactin levels than did patients who had response to therapy as determined at the end of 3 years (P < 0.001; Table 2). However, such a difference was not observed for CEA (P < 0.1; Table 2).

Table 2. Preoperative Plasma Prolactin and Carcinoembryonic Antigen in Patients With Response to Treatment and Those Who Later Had Progressive Disease*

<table>
<thead>
<tr>
<th></th>
<th>PRL (ng/ml)</th>
<th>CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients who had response to treatment</td>
<td>52</td>
<td>17.31 ± 2.69†</td>
</tr>
<tr>
<td>Patients in whom progressive disease developed</td>
<td>55</td>
<td>53.63 ± 10.39†</td>
</tr>
</tbody>
</table>

PRL, prolactin; CEA, carcinoembryonic antigen
* Data show mean ± standard error
† P < 0.001
‡ P < 0.1 (nonsignificant)

Correlation Between Prognosis and Markers

Patients with preoperative prolactin levels greater than 20.0 ng/ml plasma had significantly poorer overall survival times than did those with prolactin levels less than 20.0 ng/ml plasma. This trend was maintained even when patients were grouped according to Dukes B (chi-square, 4.73 with 1 df; P < 0.0001) or C (chi-square, 3.31 with 1 df; P < 0.001; Figs. 3 and 4). However, no difference was observed between the two patient subgroups with regard to CEA levels. In addition, when the patients were divided according to Dukes B (chi-square, 0.61 with 1 df; P < 0.5) or C (chi-square, 0.92 with 1 df, P < 0.5) the difference in survival time was statistically insignificant (Fig. 5).

Discussion

The goal of the preoperative assessment of prolactin and CEA levels in patients with colorectal cancer is:
Hyperprolactinemia and Unfavorable Prognosis/Patel et al.

Figure 4 Hyperprolactinemic patients with Dukes C (solid line) cancer had shorter overall survival times (chi-square, 3.31 with 1 df, \( P < 0.001 \)) than did patients with (broken line) prolactin levels less than 20.0 ng/ml.

define operative risk factors and to identify low-risk and high-risk groups of patients, so that high-risk patients can be followed up more intensely and treated accordingly. Initially, we had included 114 patients with Dukes A, B, or C colorectal cancer. However, the number of patients with Dukes A was only 7 during 5 years of study, so the rest of the discussion includes only patients with Dukes B or C cancer.

This study supports our previously published preliminary results of prolactin and CEA levels in patients with colorectal cancer.\(^2\) Serial measurements of prolactin and CEA levels in patients with Dukes B or C cancer showed that changes in prolactin levels correlated with the disease status in 100%, whereas CEA corresponded in only 66%. Increased prolactin levels showed a lead time of 2–3 months, and excellent correlation was observed between the increase in prolactin levels and disease recurrence. An early increase in prolactin levels is an important finding and may offer a sensitive means to predict the presence of occult disease. Similar findings were reported for breast and tongue cancer.\(^{11-15}\) Although CEA assay is well established as a single method for evaluating disease recurrence, it lacks predictive power as a reliable marker for clinical use.\(^3,16\) However, accordance between serum CEA levels and clinical status occurred in 145 of 157 (92%) patients with colorectal carcinoma.\(^17\) In this study we found that the predictive value of CEA for monitoring recurrences was too low to be useful when compared with that of prolactin. This may be related to a greater incidence of moderately or poorly differentiated tumors observed in the current study. Goslin et al.\(^18\) observed that poorly differentiated tumors are more likely to be associated with a reduced ability to produce this antigen.

The lead time observed for prolactin is important because it is believed that recurrent disease that is symptomatic or that can be detected on routine examination is likely to be advanced and usually is not curable. In addition, because prolactin correlated well with the recurrence of disease, we hypothesized that prolactin or prolactin-like molecule(s) may be secreted by the tumor. This ectopic production of prolactin may represent an early sign of the presence of occult carcinoma and is the most likely mechanism of increased prolactin levels in patients with colorectal cancer. We confirmed the presence of prolactin with the help of immunohistochemical technique in colorectal tumors (Bhatavdekar JM, Patel DD, Vora HH, Shah NG, Ghosh N, Karelia NH. Unpublished data.). Our primary hope in conducting this study was to determine whether the preoperative prolactin and CEA levels could provide valuable prognostic information in addition to the conventional prognostic features. Forty-five percent of our patients had hyperprolactinemia. In contrast, Molitch et al.\(^19\) did not notice hyperprolactinemia in a series of 215 patients with various malignancies, including those of the large intestine. Our data indicate that hyperprolactinemia is a particularly ominous sign. Hyperprolactinemic patients with Dukes B or C cancer had a shorter overall survival time than did their counterparts. From these results we postulate that hyperprolactinemia probably characterizes a subgroup of patients with aggressive colorectal cancer.

Our finding of a lack of prognostic significance of CEA levels in patients with colorectal cancer is in agreement with other reports.\(^20-22\) Among patients with Dukes C disease, we found that 24 of 35 patients with CEA levels greater than 5.0 ng/ml died, compared with 11 of 19 patients with CEA levels less than 5.0 ng/ml. We did not find a significant difference in survival time between two subgroups of CEA in patients with Dukes B or C cancer. Moertel et al.\(^23\) also observed that the use of CEA levels seems to be limited because of a lack of sensitivity in identifying individual patients with poor prognosis.

In conclusion, the changes in prolactin levels gave significantly higher predictive power than did CEA lev-
els for detection of disease recurrence in patients with Dukes B or C cancer. CEA test would seem to add little to the prognostic information obtained from standard surgical pathologic staging. We suggest the use of plasma prolactin to help identify low-risk and high-risk subgroups of patients, so that high-risk patients may be followed up more intensively and treated accordingly. Hyperprolactinemia provides additional prognostic information and may be useful as an independent predictor of short-term survival in patients with Dukes B or C colorectal cancer. We currently are investigating the expression of prolactin mRNA and prolactin gene expression by polymerase chain reaction in colorectal tumors.

References

Interrelationship of Prolactin and Its Receptor in Carcinoma of Colon and Rectum: A Preliminary Report

JYOTSNA BHATAVDEKAR, PhD, DEVENDRA PATEL, MD, FRCS, NANDITA GHOSH, MSC, HEMANGINI VORA, MSC, NEELAM SHAH, PhD, NILKAMAL KARELIA, PhD, DAMODAR BALAR, MD, PRIYA CHIKHLIKAR, MSC, AND RUCHITA DAVE, MSC


The prolactin receptors (PRLR) were correlated with circulating prolactin and various clinicopathologic parameters to investigate its prognostic value in patients with colorectal cancer. The prolactin (by radioimmunoassay) and its receptors (by radioligand method) were estimated in a total of 71 male patients with colorectal cancer enrolled at the Gujarat Cancer and Research Institute, Ahmedabad. The patients were followed for a period of 3 years. We have observed that 51% colorectal tumors were PRLR+. Significant correlation was not observed between presence/absence of PRLR and clinicopathologic variables. Dukes' D patients were lost to follow-up after 2-3 months; therefore, the results of prognostic significance were analysed only in patients with Dukes' A, B, and C (N = 64). Statistically significant difference in overall survival was not observed when the patients were subgrouped according to the presence/absence of PRLR and according to the cutoff level (i.e., 2%). PRLR+ hyperprolactinemic (Prolactin >20.0 ng/ml plasma) patients had better overall survival than that of patients with PRLR− hyperprolactinemia, although the difference was statistically nonsignificant. However, PRLR− hyperprolactinemia patients had a more unfavourable prognosis than that of their counterparts. A similar trend was observed in patients with Dukes' B and C disease.

Our preliminary study suggests an unequivocal finding, that PRLR− with concomitant hyperprolactinemia probably characterises a subgroup of patients with aggressive colorectal cancer. © 1994 Wiley-Liss, Inc.

Key Words: prolactin, prolactin receptors, colorectal cancer, prognostic significance

INTRODUCTION

During the past 2 years, we have published data on the role of tumor markers, hormones, and growth factors in patients with colorectal cancer. The results suggest that plasma prolactin (PRL) is a better marker than carcinoembryonic antigen (CEA), which can be used as a therapeutic monitor and as a prognosticator in patients with colorectal cancer [1,2]. To further strengthen our observations, this article reports the results of PRL receptors (PRLR) in patients with colorectal cancer. It is known that the action of prolactin is mediated by binding to specific membrane receptors at the cell surface. Hence, the PRLR were correlated with the circulating PRL and various clinicopathologic prognostic parameters to investigate their prognostic value.

Accepted for publication December 13, 1993
Address reprint requests to Dr J.M. Bhatavdekar, Division of Research, The Gujarat Cancer Society, Asarwa, Ahmedabad 380 016, India
© 1994 Wiley-Liss, Inc.
MATERIALS AND METHODS

Patients

A total of 71 male patients with colorectal cancer registered at our institute were included in the study. The details of clinical, pathological, biochemical data, and treatment given were noted from the case files of the patients.

Blood Collection

Blood samples were drawn prior to surgery, between 9:00 AM and 11:00 AM, to avoid diurnal variation. Blood (EDTA, disodium salt, 1–2 mg/ml) was separated within 1–2 hr and plasma aliquoted and stored at −70°C until assayed. PRL was estimated with double-antibody radioimmunoassay (RIA) kits supplied by Diagnostic Products Corporation, Los Angeles, CA, USA. The results were expressed as nanograms per milliliter (ng/ml) plasma. The assay was performed in duplicate with an intra- and interassay coefficient of variation of 3–8%. The normal range of PRL was 0.0–20.0 ng/ml plasma, and patients with PRL levels >20.0 ng/ml were considered hyperprolactinemic. The patients were followed for a period of 3 years.

Prolactin Receptor Assay

After surgery, the specimen was collected on ice. The histologically proven malignant portion was snap-frozen in liquid nitrogen and preserved at −70°C until analysis. PRLR assay was performed according to the method of Shiu et al. [3] and Patel et al. [4] using 125I-hGH (sp. act. 85–130 μCi/μg, NEN, Boston, MA, USA). For nonspecific binding o-PRL (ovine prolactin, #332, a gift from NHPP, USDA, 1 μg) was used. Specific binding was calculated as the difference between the cpm bound in the absence and in the presence of excess unlabeled hormone and expressed as percentage of the total radioactivity added to the tube. Tumors were considered positive if (1) the specific binding was more than 800 cpm [5], and (2) the cutoff level was 2% [6].

Statistical Analysis

Pearson’s chi-square statistic was used to assess the prognostic significance [7]. Significance was calculated by Fisher’s two-sided exact test [8]. The correlation coefficient (r) was calculated by Pearson’s method. Overall survival was calculated by the Kaplan-Meier method [9].

RESULTS

Sixty-five percent (46/71) patients had cancer of the rectum and 35% (25/71) patients had colon cancer. Dukes’ A, B, C, and D disease was present in 4 (6%), 33 (46%), 27 (38%), and 7 (10%) patients, respectively. PRL levels were <20.0 ng/ml plasma in 46/71 (65%) and >20.0 ng/ml plasma in 25/71 (35%) patients. Fifty-one percent (36/71) of patients had PRLR+ tumors, and 49% (35/71) of patients had PRLR− tumors. No correlation was observed between circulating PRL levels and PRLR (r = +0.12).

Correlation of Clinicopathologic Parameters and PRLR

The stratification of clinicopathologic parameters in PRLR+ and PRLR− patients is shown in Table I. None of the variables (i.e., age, site of the tumor, Dukes’ stage, and plasma PRL levels <20.0 ng/ml and >20.0 ng/ml plasma) was significantly correlated with PRLR status.

PRLR and Prognosis

In Dukes’ D patients, the overall survival was unknown, as the patients were lost to follow-up; therefore,

---

### Table I. Association Between Prolactin Receptor Status With Age, Site of the Tumor, Dukes’ Stage, and Circulating Prolactin in Patients With Colorectal Cancer

<table>
<thead>
<tr>
<th>Variables</th>
<th>PRLR+ % (N = 36)</th>
<th>PRLR− % (N = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40.0</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>&gt;40.0</td>
<td>81</td>
<td>77</td>
</tr>
<tr>
<td>Site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>67</td>
<td>63</td>
</tr>
<tr>
<td>Colon</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Dukes’ stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td>C</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Plasma prolactin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20.0 ng/ml</td>
<td>64</td>
<td>66</td>
</tr>
<tr>
<td>&gt;20.0 ng/ml</td>
<td>36</td>
<td>34</td>
</tr>
</tbody>
</table>

---

Fig. 1. Overall survival curves of PRLR+ and PRLR− patients with colorectal cancer.
TABLE II. Prolactin Levels and Overall Survival According to the Cutoff Level of PRLR (2%) in Patients With Colorectal Cancer

<table>
<thead>
<tr>
<th>PRLR</th>
<th>N</th>
<th>PRLR+ (%)</th>
<th>Prolactin+ (ng/ml plasma)</th>
<th>Overall survival+ (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2%</td>
<td>40</td>
<td>0.39 ± 0.11*</td>
<td>21.60 ± 3.47</td>
<td>25.78 ± 2.91</td>
</tr>
<tr>
<td>&gt;2%</td>
<td>24</td>
<td>3.91 ± 0.33*</td>
<td>31.07 ± 7.54</td>
<td>27.08 ± 2.71</td>
</tr>
</tbody>
</table>

*Data ±SEM.
*P < 0.0001.

TABLE III. Relationship of PRLR and Circulating Prolactin to Overall Survival in Patients With Colorectal Cancer

<table>
<thead>
<tr>
<th>PRLR</th>
<th>N</th>
<th>Cutoff level of prolactin (ng/ml)</th>
<th>Prolactin+ (ng/ml plasma)</th>
<th>Overall survival+ (mo)</th>
<th>Patient deaths (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRLR+</td>
<td>21</td>
<td>&lt;20.0</td>
<td>11.25 ± 1.10</td>
<td>28.53 ± 2.81</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&gt;20.0</td>
<td>56.82 ± 10.54</td>
<td>25.55 ± 3.95</td>
<td>46</td>
</tr>
<tr>
<td>PRLR-</td>
<td>19</td>
<td>&lt;20.0</td>
<td>8.70 ± 0.95</td>
<td>29.39 ± 2.91*</td>
<td>32†</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>&gt;20.0</td>
<td>42.69 ± 8.44</td>
<td>17.40 ± 4.05*</td>
<td>73†</td>
</tr>
</tbody>
</table>

*Data ±SEM
*P < 0.02
†X2 = 5.26, df = 1, P < 0.02

the results of prognostic significance were analysed only in those patients with Dukes' A, B, and C disease (N = 64).

Sixty-four patients were divided into two groups according to presence/absence of PRLR (i.e., PRLR+ and PRLR-). A significant difference in overall survival was not observed in these two groups (Fig. 1, χ² = 0.83, df = 1, P < 0.25). In addition to the above groups, the patients were subgrouped according to the cutoff level of PRLR (i.e., 2%). The mean concentration differed significantly between these two subgroups (P < 0.0001), whereas a significant difference in overall survival was not observed (Table II).

Relationship of PRLR and Circulating Prolactin to Overall Survival

The patients with PRLR+ and PRLR- tumors were divided according to the cutoff level of circulating PRL (20.0 ng/ml). A significant difference in overall survival was not observed in the two subgroups of patients with PRLR+ tumors. On the contrary, patients with PRLR- tumors and hyperprolactinemia had a significantly worse overall survival (Table III, P < 0.02; Fig. 2, χ² = 5.26, df = 1, P < 0.02) than that of their counterparts. Such a trend was also maintained when the patients were grouped according to Dukes' B and C disease. However, the number of patients was too small to draw any definite conclusion. Moreover, PRLR- hyperprolactinemic patients had a poorer overall survival as compared to

PRLR+ hyperprolactinemic patients, but this difference was statistically nonsignificant.

**DISCUSSION**

The relationship among hormones, growth factors, and their receptors in gastrointestinal carcinomas has been recently reviewed [10–12]. The lining of the colon is exposed to a variety of endogenous substances exerting trophic effects on gastrointestinal mucosa via autocrine/paracrine mechanisms [13]. Moreover, a number of autocrine growth factors have been associated with colorectal
cancers, for example TGF, bombesin-like peptides, and insulin-like growth factors (IGFs) [14].

With reference to our interesting and convincing observations on the role of PRL in patients with carcinoma of colon and rectum, we hypothesized that a PRL or PRL-like molecule is produced by colorectal tumors. In patients with colorectal cancer, we have observed an approximately 10-fold increase in PRL levels from tumor draining venous blood over levels in peripheral blood. Immunohistochemical localization of PRL indicated that PRL is produced by colorectal tumors. These results clearly suggest ectopic production of PRL by colorectal tumors (Bhatavdekar et al., unpublished data). The ectopically produced PRL might be acting as one of the major local peptide growth promoter via autocrine/paracrine mechanisms [15]. These mechanisms require the presence of receptors. The role of PRLRs in patients with colon and rectum is discussed here. In the present study, 51% colorectal tumors were PRLR+. However, we did not find any correlation between the presence and absence of PRLR with age, site of tumor, and stage of disease in these patients. Moreover, to evaluate the prognostic significance of these receptors, patients were subgrouped according to the two cutoff levels i.e., presence or absence as well as at >2% or <2%. Statistically significant difference in overall survival was not observed in these two subgroups of PRLR.

We have reported that hyperprolactinemia is an ominous sign in patients with Dukes, B and C colorectal cancer [2]. To substantiate our observations further, we have correlated PRLR and hyperprolactinemia. Patients with PRLR+ tumors and hyperprolactinemia had better overall survival than that of patients with PRLR− tumors and hyperprolactinemia, although the difference was statistically nonsignificant. However, we found that PRLR− hyperprolactinemic patients had unfavorable prognosis. A similar trend was observed when patients were subgrouped according to Dukes’ B and C disease. From these results, we postulate that high concentration of circulating PRL or ectopic production of PRL might be responsible for the down-regulation of PRLR. This, in turn, might be associated with enhanced metastatic potential leading to the aggressiveness of colorectal cancer. Our preliminary study suggests an unequivocal finding that PRLR− tumors and hyperprolactinemia probably characterize a subgroup of patients with aggressive colorectal cancer. We are in the process of confirming ectopic production of PRL by studying the expression of PRL mRNA in colorectal tumors. This is important because a better biologic identification of patients at high risk of subsequent metastasis will help determine which patients will benefit from new hormonal (i.e., anti-PRL) therapy.

REFERENCES
Title: ENDOGENOUS PEPTIDE AND STEROID HORMONES IN MEN WITH COLORECTAL CARCINOMA


Institution: The Gujarat Cancer Society, Asarwa, Ahmedabad-380 016, INDIA.

Affiliations: Division of Research

Mailing address: Dr. Jyotsna M. Bhatavdekar,
Division of Research,
The Gujarat Cancer Society,
Asarwa, Ahmedabad-380 016,
INDIA.

Running title: Hormones in men with colorectal cancer
Plasma FSH, LH, prolactin and serum estradiol, progesterone and testosterone were estimated by RIA in 44 male age matched controls and 69 men with colorectal carcinoma. 29% patients had colon and 71% had rectal cancer. We did not find significant difference between hormones when grouped according to site of the cancer as well as patients <40 and >40 years. In this retrospective study, there was a trend of high levels of FSH (P < 0.01), prolactin (P < 0.0001), estradiol (P < 0.05) and progesterone (P < 0.001) with concomitant low testosterone (P < 0.0001) in patients when compared with controls. These patients when grouped according to Dukes stage, the concentration of estradiol, progesterone and testosterone decreased whereas prolactin levels increased from Dukes A to D. Prolactin was elevated (>15.0 ng/ml plasma) in 61% colorectal cancer patients. From our results, we suggest that prolactin might be acting as an important regulator of both proliferation and differentiation of colorectal carcinoma.

Key Words : Peptide and Steroid Hormones, Men, Colorectal carcinoma
ENDOGENOUS PEPTIDE AND STEROID HORMONES IN MEN WITH COLORECTAL CARCINOMA

Division of Research, The Gujarat Cancer Society, Asarwa, Ahmedabad 380 016, INDIA.

I. INTRODUCTION

Colorectal cancer incidence at our institute was 9% for the year 1988-89 with an indication that the incidence is on the rise. Several lines of evidence indicate that endocrine factors may play a role in the aetiology of female colorectal cancer. The relationship among hormones, growth factors, their receptors in malignant cells has been reviewed. Since an array of trophic hormones and growth factors affects the normal bowel so dramatically, no surprise is evoked by the demonstration that same factors may act as promoters of neoplasia in carcinogen treated animals.

Our interest in colorectal carcinoma stems from the previous work and resulting data published from our division on the same subject. We reported circulating prolactin levels as an indicator of disease progression and as a better marker than CEA and as short-term prognosticator in patients with colorectal adenocarcinoma. An
early rise in prolactin in colorectal cancer patients is an important finding and may offer a sensitive means to predict the presence of recurrent disease which is often difficult to evaluate by other means. The results of prolactin suggested to us that perhaps peptide and steroid hormonal profile might be involved in the development and/or progression of colorectal cancer. Much less is known about the endogenous hormones in male patients with colorectal cancer. The present study was undertaken to fill up the lacuna in our knowledge.

II. METHODS

Between 1985 and 1991, 69 male colorectal cancer patients were enrolled. The age, sex, smoking/alcohol consumption, dietary factors, site of cancer were recorded from the cases. Diagnostic details from patients medical charts were abstracted by us. Disease was staged using Dukes classification. Pretherapeutic blood was obtained in the morning between 9.0 and 12.00 noon so that the influence of diurnal fluctuation would be avoided as much as possible. All the hormones (peptide - FSH, LH, Prolactin and steroid - Estradiol, Progesterone, Testosterone) were estimated by double antibody radiommunoassay kits procured from Diagnostic Products Co., U.S.A. The plasma (EDTA, disodium salt coated tubes, 1-2 mg/ml) and serum were separated within two hours and stored at -70 C. Assays were carried out within one month of collection. The assays were performed in duplicate with an intra- and inter-assay
variation of 3-5% and 5-8% respectively.

The normal lower and upper limits of respective hormones is mentioned below:

1. FSH : 4.5 to 20.0 mIU/ml plasma.
2. LH : undetectable to 25.0 mIU/ml plasma.
3. Prolactin : undetectable to 15.0 ng/ml plasma.
4. Estradiol : 6.6 to 36.0 pg/ml serum.
5. Progesterone : 0.05 to 0.85 ng/ml serum.
6. Testosterone : 3.0 to 9.0 ng/ml serum.

The levels above or below the range were considered elevated or decreased respectively.

Significance was calculated using the exact contingency table test for order data and Fisher's two-sided exact test. P values less than 0.05 were considered significant.

III. RESULTS

The patients' characteristics is shown in Table I. 65/69 (94%) patients were either smokers or taking alcohol regularly. 29% patients had colon cancer and 71% had rectal cancer. We also tried to find out differences in hormone levels in colon and rectal cancers but no significant difference was observed in these groups as well as according to the age i.e. younger vs older patients (<40 and >40 years). 80% patients had Dukes B and C disease. The upper age limit was same in all Dukes stages.
LH did not differ significantly between control and patients with colorectal cancers. However, FSH (P < 0.01), prolactin (P < 0.001), estradiol (P < 0.05) and progesterone (P < 0.001) were significantly high while testosterone was significantly low (P < 0.0001) in patients when compared to controls (Table II). 61% patients had prolactin above upper normal limit (>15.0 ng/ml).

Hormonal distribution according to the Dukes staging was done. No significant difference was observed in FSH and LH in all the Dukes stages while prolactin was significantly high in Dukes A to D. The estradiol and progesterone were significantly high in Dukes A when compared to D (Table III). However, intergroup variation was not observed.

IV. DISCUSSION

We have demonstrated that there was a trend of high levels of FSH, prolactin, estradiol and progesterone with concomitant low levels of testosterone in men with colorectal cancer when compared with age matched controls. Interrelationship, if any, is difficult to understand. However, the high levels of estradiol might be acting as potent stimulators of prolactin. McMichael and Potter hypothesised that endogenous estrogens increase colon cancer through increased bile acid production. Progesterone promotes differentiation of epithelial cells in the colon crypt and may also serve to maintain these differentiated cells while simultaneously inhibiting proliferation. The high levels of
estradiol and low testosterone suggests feminization but in this retrospective study, we have no parity history on men. The significantly high levels of these hormone suggests that altered hormonal levels play an important role in the pathogenesis of colorectal cancer. These hormonal alterations may rise from: (1) changes in hormone synthesis or release, (2) alterations in intracellular signal transduction mechanisms or (3) alterations at genetic level.

A highlight of our investigation is the finding that 61% patients showed hyperprolactinaemia (prolactin >15.0 ng/ml plasma) in our colorectal cancers. This is not always reflected in studies carried out in other countries. Dukes D patients had low level of prolactin so, we have estimated prolactin in the plasma and ascitic fluid collected simultaneously in a few Dukes D patients. We found significantly higher prolactin concentrations in the ascitic fluid compared to the circulating levels. On these basis we presume that prolactin, which is a low molecular weight polypeptide (approx. 23,000 dalton) easily escapes into the ascitis from the circulation or the lymphatics. Moreover, we also have estimated prolactin from tumor draining veins and observed nearly 10-15 fold increase in prolactin concentration when compared with peripheral levels (Bhatavdekar et al., unpublished data). This lead us to believe that prolactin alongwith other hormones probably acts as an important regulator both of proliferation and differentiation in colorectal carcinomas. Additional studies are underway in this division to find...
out the significance of prolactin receptors and prolactin mRNA in the development and progression of colorectal carcinoma.

These results complement our clinical trial with antiprolactin drug in advanced breast cancer patients. Thus, in view of the evidence produced by the current study, there appears some justification in at least a subgroup, perhaps small, which would benefit of a new "endocrine" treatment in patients who are hyperprolactinaemic either at presentation or subsequently.

ACKNOWLEDGEMENTS

The research work was supported by the Indian Council of Medical Research, (# 8704250), New Delhi, India. The authors are thankful to Dr. N. L. Patel, Director, Gujarat Cancer and Research Institute for providing necessary facilities.

REFERENCES


### TABLE I
CHARACTERISTICS OF COLORECTAL PATIENTS.

<table>
<thead>
<tr>
<th>Dukes stage</th>
<th>%</th>
<th>N</th>
<th>Age</th>
<th>Colon</th>
<th>Rectum</th>
<th>% Elevation of Prolactin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>6</td>
<td>38-75</td>
<td>2</td>
<td>4</td>
<td>67%</td>
</tr>
<tr>
<td>B</td>
<td>42</td>
<td>29</td>
<td>32-70</td>
<td>10</td>
<td>19</td>
<td>66%</td>
</tr>
<tr>
<td>C</td>
<td>38</td>
<td>26</td>
<td>17-70</td>
<td>6</td>
<td>20</td>
<td>57%</td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td>8</td>
<td>24-74</td>
<td>2</td>
<td>6</td>
<td>50%</td>
</tr>
</tbody>
</table>
### TABLE II

**HORMONAL LEVELS IN CONTROL AND COLORECTAL CANCER PATIENTS**

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=44)</th>
<th>Colorectal patients (N=69)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH</strong> (mIU/ml)</td>
<td>8.72 ± 0.91</td>
<td>13.47 ± 1.50</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>% Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LH</strong> (mIU/ml)</td>
<td>16.03 ± 0.77</td>
<td>20.57 ± 4.01</td>
<td>NS</td>
</tr>
<tr>
<td>% Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prolactin</strong> (ng/ml)</td>
<td>8.89 ± 0.69</td>
<td>30.96 ± 4.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Estradiol</strong> (pg/ml)</td>
<td>22.24 ± 3.72</td>
<td>37.81 ± 6.28</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>% Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Progesterone</strong> (ng/ml)</td>
<td>00.06 ± 0.02</td>
<td>00.59 ± 0.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>% Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Testosterone</strong> (ng/ml)</td>
<td>6.42 ± 0.22</td>
<td>3.69 ± 0.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>% Reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% Elevation = Above upper normal limit
% Reduction = Below lower normal limit
### TABLE III
DISTRIBUTION OF HORMONES ACCORDING TO DUKES STAGE

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Dukes stage</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>29</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td></td>
<td>10.46 ± 0.37</td>
<td>14.18 ± 0.38</td>
<td>13.97 ± 0.81</td>
<td>11.51 ± 0.40</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>1.0 - 18</td>
<td>1.0 - 58</td>
<td>0.0 - 51</td>
<td>1.62 - 34</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td></td>
<td>10.71 ± 0.39</td>
<td>23.21 ± 0.92</td>
<td>12.68 ± 0.85</td>
<td>43.91 ± 25.98</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>5.0 - 29</td>
<td>2.55 - 175</td>
<td>0.0 - 44</td>
<td>1.0 ± 205</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td></td>
<td>19.78 ± 0.49</td>
<td>35.13 ± 0.49</td>
<td>33.51 ± 0.18</td>
<td>15.95 ± 0.59</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>4.8 - 39</td>
<td>2.5 - 165</td>
<td>1.22 - 155</td>
<td>1.05 - 40</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td></td>
<td>57.96 ± 20.48</td>
<td>40.64 ± 10.31</td>
<td>34.19 ± 10.52</td>
<td>24.20 ± 14.94</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>19.0 - 155</td>
<td>0.0 - 235</td>
<td>0.0 - 260</td>
<td>1.0 - 124</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td></td>
<td>0.84 ± 0.57</td>
<td>0.59 ± 0.21</td>
<td>0.64 ± 0.20</td>
<td>0.33 ± 0.19</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0.0 - 3.15</td>
<td>0.0 - 4.4</td>
<td>0.0 - 2.4</td>
<td>0.1 - 1.6</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td></td>
<td>4.11 ± 0.96</td>
<td>3.75 ± 0.32</td>
<td>3.76 ± 0.42</td>
<td>2.97 ± 0.58</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>2.1 - 8.25</td>
<td>0.48 - 6.0</td>
<td>0.53 - 8.2</td>
<td>0.77 - 5.8</td>
</tr>
</tbody>
</table>

P value: Prolactin Dukes B vs D <0.05